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博　士　学　位　论　文

中国深圳海域及九龙江口细菌生态特征研究

Studies on the Characteristics of Bacterial Ecology in Shenzhen Coastal Waters and Jiulong River Estuary, China

陈明霞

指导教师姓名：郑天凌　教授

李和阳　副研究员

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目　　录

CONTENTS

摘　要

海洋弧菌是海洋微食物环的重要组成部分，对海洋环境营养物的循环起着非常重要的作用。此外，弧菌还是人类及水生动物（包括养殖动物）的重要致病菌。对弧菌及其相关类群的种类组成、数量分布、与环境相关关系以及耐药性的研究是环境细菌学研究的重要组成部分，其研究对环境健康、疾病防治及生态安全等方面具有重要意义。本文运用传统的分离培养方法和现代的分子分类检测方法，对深圳海域及九龙江口弧菌（包括可培养、未培养的）的生态分布及其与环境相关关系进行研究。

研究内容包括两大部分：

1. 深圳海域弧菌种类组成、数量分布及其与环境相互关系研究

2. 九龙江口 TCBS 类群(生长于 TCBS 琼脂培养基上的细菌类群)的分布及其耐药性研究

主要研究结果包括：

1. 深圳海域可培养弧菌数量分布具有季节性。东部海域弧菌数量春季（2008 年 4 月）（范围为 5.10×10^2-4.40×10^4 CFU/mL，平均为 1.50×10^4 CFU/mL）高于秋季（2008 年 9 月）（范围为 1.41×10^2- 2.57×10^3 CFU/mL，平均为 8.89×10^2 CFU/mL）；西部海域秋季（范围为 0-1.56×10^3 CFU/mL，平均为 5.09×10^2 CFU/mL）略高于春季（范围为 0-6.62×10^2 CFU/mL，平均为 2.66×10^2 CFU/mL）；弧菌数量高发区出现在 4 月份大亚湾（4.40×10^4 CFU/mL）。弧菌分布与温度、有机物浓度及盐度等相关，各环境因素互相交联，其相互关系复杂。

2. 珠江口盐度低于 11 的站位未检测到可培养的弧菌，盐度高于 11 的站位，可培养弧菌数量随盐度增加而增大。九龙江口盐度较低的上游区域沉积物中未检测到可培养弧菌，而在盐度较高的河口下游区域有弧菌分布，而且其分布比例随盐度增加而增加。

3. 深圳海域清洁水域（YMK001 站位和 GDN064 站位）发现有大量的弧菌存在，且数量高于污染严重的海域（包括深圳湾站位及珠江口下游站位），是弧菌数量的高发区。

4. 深圳海域春季可培养弧菌有 *V. gigantis*、*V. splendidus*、*V. alginolyticus*、*V. cyclitrophicus*、*V. rotiferianus*、*V. mytili* 、*V. natriegens*、*V. parahaemolyticus* 和 *V.*

harveyi 的类似种，其中主要优势类群是 *V. gigantis* 类似种、*V. alginolyticus* 的类似种和 *V. splendidus* 的类似种；秋季分布有 *V. alginolyticus*、*V. natriegens*、*V. mytili*、*V. splendidus* 和 *V. fortis* 的类似种，其中主要优势类群是 *V. alginolyticus* 类似种和 *V. natriegens* 类似种。各类优势弧菌分布都具有季节性：*V. alginolyticus* 类似种是两个季节的优势类群，其平均数量春季高于秋季；春季特有的优势类群有 *V. gigantis* 类似种和 *V. splendidus* 类似种，秋季特有的优势类群有 *V. natriegens* 类似种。

5. 未培养弧菌种类包括：*V. pomeroyi*、*V. sinaloensis*、*V. splendidus*、*V. pelagius*、*V. cholerae*五种弧菌的类似种，其中*V. pomeroyi*类似种是两个月份的优势弧菌类群；*V. sinaloensis*类似种主要发生于9月份水体温度较高的季节，*V. splendidus*类似种主要发生于4月份水体温度较低的季节。

6. 九龙江口沉积物（2009年12月）的可培养弧菌类群以*V. parahaemolyticus*为主。此外还大量分布有其他TCBS类群，如*Shewanella*、*Pseudoalteromonas*、*Pseudomonas*、*Aeromonas*、*Bacillus*属细菌。深圳海域也同样具有大量非弧菌的TCBS类群存在。弧菌占TCBS菌株总数的比例因不同站位、不同季节而有很大的变化，其比例为0-100%。盐度较高的区域，弧菌数量与TCBS菌群数呈直接正相关，盐度较低的区域弧菌数量与TCBS菌群数无关或关系不明显。

7. 九龙江口TCBS菌株53%对氨苄青霉素耐药，59%对头孢唑啉耐药，72%对头孢噻吩耐药，1%对庆大霉素耐药，4%对氟哌酸耐药，3%对四环素耐药，7%对链霉素耐药。有些菌株为3联、4联或6联耐药菌株。

关键词：深圳海域，九龙江口，弧菌种类组成及数量分布，TCBS类群，耐药性

Abstract

Vibrios are dominant in the marine environment and play a key role in nutrient cycling in aquatic environments by taking up dissolved organic matter and producing kinds of enzymes. Otherwhile, vibrios are important bacterial pathogens for humans and marine animals, because of which, more and more researchs focus on the composition of vibrios and the relationship with the environmental factors.

Research contents:

1. Composition and distribution of vibrios and their correlation with the environmental factors in Shenzhen coastal waters.

2. Antibiotic resistance and distribution of TCBS groups from sediments of Jiulong River estuary.

Results:

1. Seasonal variations of culturable vibrios in Shenzhen coastal waters were obvious. Within the east coastal waters, the abundance of marine vibrios in Spring (April 2008) (range from 5.10×10^2 CFU/mL to 4.40×10^4 CFU/mL，with an mean value of 1.50×10^4 CFU/mL) was higher than that in Autumn (September 2008) (range from 1.41×10^2 CFU/mL to 2.57×10^3 CFU/mL，with an mean value of 8.89×10^2 CFU/mL), while within the west coastal waters, the abundance of marine vibrios in Autumn (range from 0 CFU/mL to 1.56×10^3 CFU/mL，with an mean value of 5.09×10^2 CFU/mL) was higher than that in Spring (range from 0 CFU/mL to 6.62×10^2 CFU/mL，with an mean value of 2.66×10^2 CFU/mL). The highest number was detected in Daya Bay in Spring (4.40×10^4 CFU/mL). The distribution of vibrios were mainly controlled by temperature, salinity, pH and organic concentration, et al. The relationships between vibrios and environmental factors were complicated and varied depending on different species, locations and seasons.

2. There were no culturable vibrios detected at some Zhujiang river estuary stations where salinity was under 11. Also, There were no culturable vibrios detected in the fresh or brackish stations without the more saline stations of Jiulong river estuary. The results showed that salinity played an important role in the distribution of vibrios

which indicated that vibrios are autochthonous of the marine environment.

3. Vibrios abundance in healthy waters (stations YMK001 and GDN064) was higher than that in badly polluted waters (stations GDN053, GDN057 and GDN063), which showed that vibrios were the natural and important group in the marine ecosystem.

4. In Spring, culturable vibrios in the Shenzhen coastal waters were composed of *V. gigantis*-like species, *V. splendidus*-like species, *V. alginolyticus*-like species, *V. cyclitrophicus*-like species, *V. rotiferianus*-like species, *V. mytili*-like species, *V. natriegens*-like species, *V. parahaemolyticus* -like species and *V. harveyi*-like species, among which *V. gigantis*-like species, *V. alginolyticus*-like species and *V. splendidus*-like species were the three dominant groups. In Autumn, vibrios in the same waters were composed of *V. alginolyticus*-like species, *V. natriegens*-like species, *V. mytili*-like species, *V. splendidus*-like species and *V. fortis*-like species, among which *V. alginolyticus*-like species and *V. natriegens*-like species were the dominant groups. Seasonal variations of each dominant *Vibrio* sp. in the coastal waters was obvious: *V. alginolyticus*-like species was the dominant group in both seasons, the average number of which in April was higher than that in September; *V. gigantis*-like species and *V. splendidus*-like species were dominant in April with lower temperature, while *V. natriegens*-like species was dominant in September with higher temperature.

5. Uncultured vibrios in the Shenzhen coastal waters were composed of *V. pomeroyi*-like species, *V. sinaloensis*-like species, *V. splendidus*-like species, *V. pelagius*-like species and *V. cholerae*-like species, among which *V. pomeroyi*-like species was the main dominant group in both seasons, *V. sinaloensis*-like species was dominant in Autumn with higher temperature, and *V. splendidus*-like species was dominant in Spring with lower temperature. The results indicated that there still were a large number of vibrios in VBNC state.

6. *V. parahaemolyticus*-like species was the only culturable vibrios detected in the sediment of Jiulong River estuary in December 2009. Besides ot this, the TCBS groups were composed of *Shewanella* sp., *Pseudoalteromonas* sp., *Pseudomonas* sp., *Aeromonas* sp. and *Bacillus* sp.. And there were non-vibrios specieses detected in

Shenzhen coastal waters with TCBS agar too. The ratio of the number of vibrios to TCBS strains changed from 0% to 100% depending on different locations and seasons. The number of vibrios showed a posive correlation with that of TCBS strains in more saline sea waters, and no correlation in fresh or brackish waters.

7. The percentage of resistant strains to ampicillin, cefazolin, cephalothin, norfloxacin, gentamicin, tetracycline and streptomycin was 53%, 59%, 72%, 4%, 1%, 3% and 7% respectively. Some strains resisted to 3, 4 or 6 antibiotics.

Key words: Shenzhen coastal waters, Jiulong River estuary, composition and distribution of vibrios, TCBS groups, antibiotic resistance